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DYSENTERY AND ALLIED BACILLI

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In France we not infrequently experienced difficulties in growing dysentery bacilli and work was therefore begun (1) to differentiate the true dysentery bacilli, which are universally recognized as pathogenic, from the atypical or dysentery-like organisms (*B. ambiguus*, *B. alkalescens*, and *B. dispar*) many strains of which are nonpathogenic and whose etiologic significance is questionable; (2) to devise a more dependable, and if possible more simple medium, than the nutrient agar (phenolphthalein titration) for the isolation of dysentery bacilli.

The nomenclature in the group of dysentery bacilli has become quite confused. In this paper the following will be adhered to: *B. dys. Shiga* corresponds to the original Shiga-Kruse mannite negative type. The term *B. flexneri* includes both the *B. dys. Flexner* and *Y* types, and when possible it will be qualified with the race of the strain, such as *V*, *W*, *X*, *Y* or *Z*. The terms *B. dys. Flexner* and *B. dys. Y* are used in their old significance.

Serologic tests and studies on classification were beyond the scope of the investigation. Agglutination with stock Flexner and *Y* serums were carried out with 59 cultures. Acid production in a number of sugars and other fermentable substances, as well as the reactions in milk and the indol test, were observed on all the stains.

A total of 111 cultures were considered in this study. These were distributed as follows: *B. dys. Shiga*, 17; *B. ambiguus*, 5; *B. flexneri*, 60; *B. alkalescens*, 12; *B. dispar*, 11; miscellaneous, 6.

The Shiga cultures, with one exception, were stock strains found at the Central Medical Laboratory or the Army Medical School; several were duplicates.

The ambiguous strains included 3 (67, 68 and 69) from Dr. Andrews, St. Bartholomew's Hospital, London. One (4) was found at the Central Medical Laboratory marked *B. dys. Shiga Fletcher vaccine stain*, and another (101) obtained from the Army Medical School and probably a duplicate of (4), was marked *B. dys. Shiga Fletcher 1*. Serologic tests were not made with (101). The other (4) failed to agglutinate with several Shiga serums, and as both were positive for indol they are here considered as *B. ambiguus*.

The 60 cultures of *B. flexneri* include strains isolated during the war and also standard stock cultures. Included in this group are the old Flexner and *Y* types and authentic strains of the English groups *V*, *W*, *X*, *Y*, *Z*, *VZ* and *WX*, which were sent me by Dr. Andrews.

There were 12 strains of *B. alkalescens* and 11 of *B. dispar*. These were received from Dr. Andrews or freshly isolated in laboratories of the A. E. F.

Of the 6 miscellaneous strains two (37 and 57) were marked *B. ambiguus*. They produced a green fluorescence in broth and on gelatin and were so different culturally from the strains of *B. ambiguus* received from Dr. Andrews that it seems they should not be considered as of the same group. Two cultures (48 and 108) were marked *B. dys. Sonne*. They did not agglutinate with the Flexner or Y serums available. Lactose was fermented with acid formation and then became alkaline. Milk was turned acid but not coagulated. These cultures resemble markedly some of the *B. dispar* of Andrews, at least culturally. One strain, 3, supposedly a Shiga, produced acid from sucrose and gave indol. It was not agglutinated with a Shiga serum. Another strain, 97, differed from all of the other cultures studied in that it fermented the glucoside salicin with a strong acidity in 24 hours.

AGGLUTINATION WITH FLEXNER AND Y SERUMS

Agglutination was made with living 24-hour broth cultures of 59 strains. The strains of *B. dys. Shiga*, *B. alkalescens*, and *B. dispar* were not agglutinated by either of the serums. *B. dys. Sonne* (48) and one of the English *B. flexneri* Z race (53), were also not agglutinated. It was noticed that the Z and X races of *B. flexneri* were only agglutinated in the low dilutions, and that (13 and 38) the original Mt. Desert Y and the Oxford Y strain, respectively, were not agglutinated even in 1:100 by the Y serum employed. From these observations it appears quite evident that what is regarded as the Y type of dysentery in different laboratories is not of the same serologic group.

BIOCHEMICAL REACTIONS (TABLE 1)

All strains were gram-negative short rods, and nonmotile as determined in semisolid agar (0.5% agar in broth).

TABLE 1
ACID PRODUCTION AND INDOL (PERCENTAGE OF POSITIVE REACTIONS) BY DYSENTERY AND CLOSELY ALLIED BACILLI

Organism	No. of Strains	Mannitol	Lactose	Glycerol*	Dextrin	Dulcitol	Sucrose	Xylose	Raffinose	Rhamnose	Indol
<i>B. dys. Shiga</i>	17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>B. ambiguus</i>	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
<i>B. dys. flexneri</i> †.....	59	100.0	0.0	0.0*	40.0	0.0	64.4	0.0	79.7	16.9	83.1
<i>B. alkalescens</i>	12	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	100.0
<i>B. dispar</i>	11	100.0	100.0	81.3	0.0	18.2	81.8	81.8	91.9	100.0	81.8

* Slight acidity in 5-7 days but more alkaline than P_H 7.0.

† Includes all mannite fermenting true dysentery bacilli.

Tests for acid production were made on glucose, mannitol, lactose, glycerol, sucrose, dextrin, arabinose, dulcitol, rhamnose, xylose, raffinose and salicin. The medium employed consisted of 1% peptone, and 0.4% dipotassium phosphate with 1% of the test material. The rosolic acid-china blue mixture of Bronfenbrenner was the indicator. Incubation was at the body temperature, and observations were made daily for 7 days.

The indol reaction was determined from peptone water after 5 days' incubation by the nitroso-indol reaction. Litmus milk was observed for 13 days.

Table 1 indicates that the 5 main types of dysentery and dysentery-like organism may be readily differentiated by fermentation and indol reactions

Thus *B. dys.* Shiga and *B. ambiguus* may be distinguished from the others (*B. flexneri*, *B. alkalescens*, and *B. dispar*) by the inability of the former to give acid from the alcohol mannitol. They differ from each other in that *B. ambiguus* forms indol and ferments rhamnose.

B. flexneri may be differentiated in a large proportion of instances from *B. alkalescens* and *B. dispar* by the reaction in glycerol and xylose. None of the Flexner strains produced acid from xylose, whereas this substance was fermented vigorously by 21 of 23 strains of *B. alkalescens* and *B. dispar*. Differentiation by glycerol fermentation was not so distinct, as a number of the Flexner strains produced a small amount of acid. Quantitative studies showed that this acidity was never beyond the true neutral point P_H 7.0. in 5 days. With the indicator employed, however, the results might be confusing in inexperienced hands.

B. flexneri differs also from *B. alkalescens* and *B. dispar* in the milk reaction. The former produces a faint acidity in litmus milk, which reverts very slowly, if at all, to a neutral reaction in from 10-13 days. *B. alkalescens*, on the other hand, reverts relatively rapidly, from 4-8 days, to a distinct alkaline reaction, while *B. dispar* becomes progressively more acid, eventually coagulating the medium. Unfortunately the milk reaction has not given concordant results in the hands of different observers, many recording distinct alkalinity and others coagulating with true dysentery strains of *B. flexneri* type.

It remains to differentiate *B. alkalescens* from *B. dispar*. The milk reaction has been referred to. The objectionable features of this reaction are the variability of different batches of milk and slowness of the test. The lactose fermentation of *B. dispar*, although distinct, is often long-delayed. Table 1 shows that although there is some overlapping, the two organisms are markedly different when groups of characters rather than single reactions are considered. Thus *B. alkalescens* does not form acid from lactose, sucrose or raffinose, but attacks dulcitol vigorously, while *B. dispar* rarely ferments dulcitol, but does form acid from lactose and most always from sucrose (81.8%) and raffinose (91.9%). *B. alkalescens* seems to be a very homogenous group. *B. dispar* probably consists of several varieties. The indol-negative, xylose-negative variety of *B. dispar* corresponds culturally to the strain isolated by Sonne in Denmark.

VARIETIES OF *B. FLEXNERI*

A number of subdivisions of the mannite fermenting dysentery strains on serologic and biochemical reactions have been proposed in the past. The probable untenability of *B. dys.* Y as distinguished from *B. dys.* Flexner has already been referred to. The differentiation of *B. flexneri* by the English War Committee as determined by careful absorption tests into V, W, X, Y and Z races appears much more acceptable and desirable.

The value of differentiation of this group on fermentation reactions has fallen into disrepute of late. Thus the fermentation of maltose, sucrose and dextrin, which were formerly emphasized as differentiating varieties of mannite fermenting dysentery strains, is about to be discarded. Maltose was not employed in this study as it was considered unreliable on account of the difficulty in obtaining a product entirely free from glucose, and the ease with which it decomposes on sterilization. Of the tests tried with 59 strains of *B. Flexneri* the following positive results were obtained with substances that might be of value for subdivision: sucrose, 64.4%; dextrin, 40%; rhamnose, 16.9%; raffinose, 79.7%; and indol, 83.1%. The correlation coefficients for each

pair of characters is given in table 2¹ which shows rhamnose correlates best with the other characters. Subdividing on rhamnose, two groups are obtained as follows:

	Strains	Sucrose	Percent Dextrin	Positive Raffinose	Indol
Rhamnose +	10	80	70	0	100
Rhamnose -	49	60	32	94	78

TABLE 2
CORRELATION COEFFICIENTS FOR FERMENTATIVE CHARACTERS

	Sucrose	Dextrin	Rhamnose	Raffinose	Indol
Sucrose.....	+0.62	+0.43	-0.06	+0.35
Dextrin.....	+0.62	+0.65	-0.46	+0.50
Rhamnose.....	+0.43	+0.65	-1.00	+1.00
Raffinose.....	-0.06	-0.46	-1.00	-0.45
Indol.....	+0.35	+0.50	+1.00	-0.45

Raffinose fermentation is particularly interesting. Of 30 strains in the rhamnose-negative subgroup which fermented sucrose, all attacked raffinose; but, of 8 sucrose fermenters in the rhamnose-positive subgroup none attacked the trisaccharid. The source of the 10 rhamnose fermenting strains was: Strain 26 was isolated at the Cent. Med. Dept. Lab. from a patient and diagnosed as probably B. dys. Y; (60 and 61) were isolated at Lab. 1, A. E. F., from a patient and carrier, respectively, and reported as B. dys. Flexner and B. dys. Y. and sent in for further identification. As the foregoing diagnoses were based merely on the two serums available—Flexner and Y—the designations should not be accepted as final. It would be desirable to know to which race of Flexner bacilli they belong. The remaining 7 strains were received from Dr. Andrews and Dr. Inman of London. One, 94, was labelled B. flexneri Y race which is a sort of composite of the V, W, X and Z races. The other 6 strains were all B. flexneri Z race. Thus there seems to be a correlation between rhamnose fermentation and the Z race of B. flexneri. If subdivision is to be made at all on fermentation reactions, then it appears that rhamnose would be the logical choice.

ACID PRODUCTION FROM GLUCOSE

In order to devise a medium for the differentiation of B. alkalescens and B. dispar from the other dysentery or dysentery-like strains, the effects of various constituents of a selected medium on the rate of acid production and reversion were studied. Ten cultures, 2 B. dys. Shiga, 2 B. dys. Y, 2 B. dys. Flexner, 2 B. alkalescens, and 2 B. dispar were chosen for study.

Concentration of glucose.—The medium consisted merely of peptone (Difco) 1% dipotassium phosphate 0.4%, and glucose in varying amounts 0.0 to 0.5%, prepared in the following manner: To 1,000 cc of distilled water in a flask was added 10 gm. of peptone, 4 gm. of dipotassium phosphate, and the flask was then heated until the contents were dissolved (about 20-30 minutes). The medium was then filtered through paper and enough of a freshly prepared 10% glucose solution was added to give the desired concentration of the carbohydrate. The medium was tubed (about 20-25 cc) and sterilized in the autoclave 10 minutes at 10 pounds, after which it was incubated to eliminate unsterile tubes.

¹ See Levine, Jour. Infect. Dis., 1918, 3, p. 253.

Inoculation was made with 0.1 cc of a 24-hour broth culture with incubation at body temperature.

H-ion concentration was determined daily for 4 days by withdrawing 1 cc of the culture into 4 cc of neutral distilled water (P_H 7.0) in a clean, flat bottomed test tube, and after adding the required amount of an appropriate indicator, the color was matched with H-ion standards. Great difficulty was encountered in obtaining neutral distilled water in the laboratory in France. It was found, however, that the error introduced by the neutralization of ordinary distilled water with a small amount of sodium hydroxid was only about 0.1, which was within the limits of error in reading. Such neutralized water had to be freshly prepared and quickly utilized.

INCUBATION DAYS.

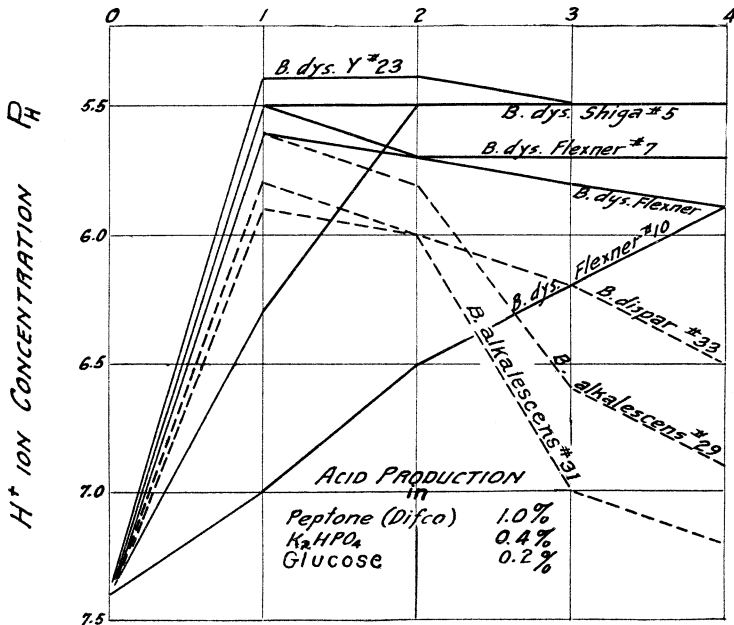


Chart 1.—Effects of peptone, dipotassium phosphate and glucose on the rate of acid production and reversion.

It was concluded (1) that with 1.0% peptone and 0.4% dipotassium phosphate, the employment of 0.3% or more glucose was undesirable for the purpose of differentiating *B. alkalescens* and *B. dispar* from the true dysentery bacilli; (2) that in the absence of glucose *B. alkalescens* and *B. dispar* form alkali more rapidly than the true dysentery strains (*Shiga* and *B. flexneri*); (3) that with 0.1% glucose there is reversion among the true dysentery strains, but *B. alkalescens* and *B. dispar* revert much more rapidly; (4) that with 0.2% glucose reversion among the true dysentery cultures was greatly inhibited, whereas *B. alkalescens* and *B. dispar* showed a marked alkali production after the primary acidity, as is shown in chart 1.

Concentration of Peptone.—The following experiment was made in Washington to determine the effect of the concentration of peptone on acid production and reversion:

Three batches of medium (0.2% glucose, 0.4% dipotassium phosphate, and 1.0%, 1.5% and 2.0% peptone respectively) were prepared as described; 7 cc portions were placed in tubes, autoclaved at 10 pounds for 10 minutes and incubated for 48 hours to eliminate unsterile tubes.

Seven tubes of each medium were inoculated from 24-hour cultures of organisms and incubated at 37 C.

Acidity determinations were made by the comparator in place of the dilution method previously described. Two duplicate cultures were taken and to one was added 0.3 cc of an appropriate indicator and the color matched with standards, the duplicate tube being employed to correct the error due to the color and turbidity of the culture medium in the comparator test. This tube was reincubated and employed for this purpose in acidity determinations on subsequent days.

The concentration of peptone did not influence acidity production nor reversion of the true dysentery bacilli nor of *B. ambiguus*, but that with *B. alkalescens* and *B. dispar* reversion was much more rapid with 1.5% peptone than when 1.0% peptone was employed. Increasing the concentration to 2.0% did not further increase the rate of reversion.

Comparing the results with 1.0% peptone with those previously obtained in the original experiment in France, reversion was somewhat delayed in the new series. Although an adequate explanation is not available, it is felt that the difference is probably due to a difference in the actual concentration of glucose. The glucose available overseas was probably not thoroughly anhydrous.

Aeration seems to increase the rate of alkali production, after the primary acidity, in the case of *B. alkalescens* and *B. dispar*.

To determine whether the differentiation indicated in the quantitative observations could be applied qualitatively, each organism was inoculated in duplicate into the peptone phosphate medium containing as indicators 1% of a 0.5% phenol-red and 1% of a 0.2% brom-cresol-purple, respectively, and incubated at 37 C. Records of acidity were made daily.

With exception of (37 and 57), which have been referred to as probably misplaced in this group, and which remained alkaline throughout the experiment, all other cultures were distinctly acid to both indicators after 24 hours' incubation. With brom-cresol-purple as the indicator, all cultures of *B. alkalescens* and *B. dispar* showed reversion to distinct purple-blue color, as did also one of the *B. dys. Sonne* after 3 days' incubation. The true dysentery strains and *B. ambiguus* were yellow or brownish in color. On further incubation (6 days), the other strain of *B. dys. Sonne* and one *B. flexneri* became distinctly alkaline and a number of the true dysentery cultures began to show some reversion, thus obscuring, though not eliminating, the differentiation.

With phenol-red, on the other hand, all cultures of *B. dys. Shiga* and *B. ambiguus*, and all but one of *B. flexneri* were distinctly acid for 6 days. The 12 *B. alkalescens* strains were distinctly alkaline. Two of the 11 *B. dispar* were neutral, the others distinctly alkaline. One *B. dys. Sonne* was neutral and another alkaline.

Rate of Acid Production.—It was observed that glucose was attacked more rapidly by *B. alkalescens* and *B. dispar* than by the other organisms of this collection. Inoculation was from 24-hour broth cultures (0.1 cc to 30 cc of

medium) and H-ion determinations were made by the dilution method. In chart 2 the data are shown graphically.

The rate of acid production was observed qualitatively by the use of brom-cresol-purple and in some instances with the china-blue rosolic acid indicator. Inoculation was made from 24-hour agar slants; incubation was at 37 C. in the ordinary manner; acidity was recorded after 6 hours. At this time all strains of *B. alkalescens* and *B. dispar*, one *B. flexneri* and the 2 *B. dys.* Sonne were distinctly acid as indicated by a distinct or dirty yellow with brom-cresol-purple. All other strains produced acid less rapidly. They showed a distinct purple (more alkaline than P_H 6.3) with brom-cresol-purple and with the china-blue mixture were colorless or light blue.

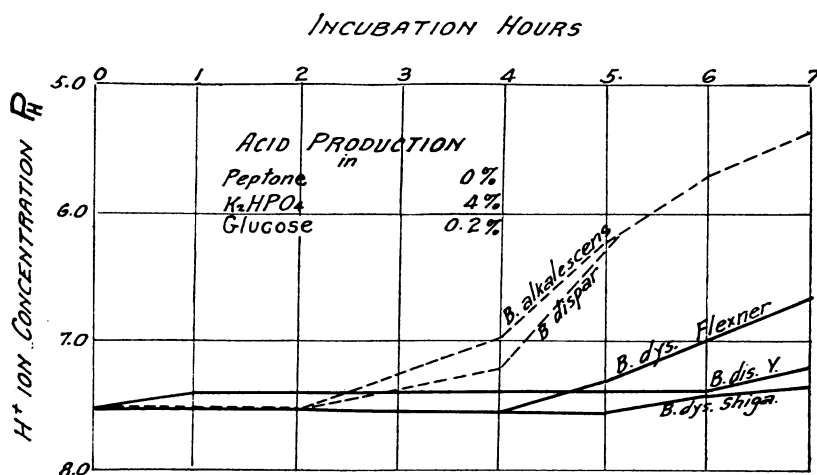


Chart 2.—Rate of acid production: Inoculations made from 24-hour broth cultures and H-ion determinations made by dilution method.

It may be concluded from the observations of glucose fermentation that *B. alkalescens* and *B. dispar* produce acid more rapidly and then revert to a distinctly alkaline reaction that may be indicated qualitatively by phenol-red or brom-cresol-purple. The use of brom-cresol-purple, however, would require experience and care, whereas phenol-red necessitates a prolonged period of incubation. The most desirable indicator for qualitative differentiation would be one which changes at P_H 6.5 showing a distinct coloration on the alkaline side.

A SIMPLIFIED SOLID MEDIUM FOR GROWTH AND ISOLATION OF DYSENTERY BACILLI

After a number of preliminary experiments it was found that by the addition of a small amount of glucose (0.03-0.05) to peptone-phosphate agar, growths as luxuriant, if not more so, than on nutrient agar could be obtained. As facilities for determination of the optimum H-ion concentration were not available at the time in France a series of experiments were carried out to determine the optimum concentration of dipotassium phosphate for growth of dysentery bacilli in a medium not requiring any further adjustment of reaction.

The medium consisted of 1.0% peptone, 0.1% glucose and 0.2 to 0.7% phosphate. Inoculation of the agar plates was made from a 24-hour broth culture. A concentration of 0.4-0.5% of the phosphate gave best results with 6 cultures examined. It is interesting in this connection to note that the titratable acidity with phenolphthalein was in each instance $+0.7\%$. The H-ion concentration was much more varied, probably 7.1 with the 0.2% of the phosphate and 7.8 with the 0.7% of the buffer salt, as indicated by subsequent experiments.

The influence of the H-ion concentration on the growth of the dysentery bacilli seems marked on solid medium. It has been my experience that in liquid medium the effect of the H-ion concentration is not so evident.

Experiments on the effect of the concentration of dipotassium phosphate repeated with 39 strains using 3 concentrations of the salt (0.2, 0.45 and 0.7%, respectively), showed that:

1. The phenolphthalein titration is a poor index of the true acidity of the medium. The variation in the titratable acidity was close to the limit of experimental error, whereas the difference in H-ion concentration with the different quantities of phosphate was marked and distinct.

2. The optimum reaction is not the same for all strains of dysentery. Two (5.1%) grew best with the largest quantity of phosphate, four (10.2%) with the least amount of phosphate and twelve (30.7%) show their optimum growth when 0.45% of dipotassium phosphate was used. Seventeen (43.6%) did equally well on all of the 3 mediums. Considering all cultures, we find 33 (84.7%) to have done as well or better with 0.45% phosphate than on either of the other concentrations of this salt. The H-ion concentration with 0.4% of the phosphate is generally 7.4 or 7.5. This quantity was selected as probably the most reliable and desirable.

Choice of Indicator.—A distinct and noninhibitory indicator is an important adjunct to the successful isolation of dysentery bacilli. It was hoped that the eosin and methylene-blue combination of Holt, Harris and Teague, which was found so valuable in water work, might be successfully employed, particularly as it was reputed to be noninhibitory. Thirty-nine strains of dysentery bacilli were inoculated on agar with and without the indicator from a 24-hour peptone phosphate culture.

The composition of the medium was:

Agar	1.5%
Peptone	1.0%
Dipotassium phosphate	0.4%
Glucose	0.1%
Indicator per 100 c c of above	
Eosin 2% yellowish aq.....	2.0 c c
Methylene-blue 0.5% aq.....	2.0 c c
(The P_H of this medium was 7.5).	

B. dys. Shiga was markedly inhibited. A slight growth was observed on prolonged incubation (48-72 hours). Sixteen, or 50%, of the mannite fermenting dysentery strain were partially inhibited.

Of a number of indicators tried, the china-blue rosolic acid mixture was found to be the least inhibitory when working with pure cultures. Similar results were obtained with artificial suspensions of dysentery organisms in normal stools.

SUMMARY AND CONCLUSIONS

Observations made on 111 strains of dysentery and dysentery-like organisms indicate:

1. The strains of *B. dysenteriae* Y used in different laboratories are not of the same serologic group.

2. The main groups of the dysentery and closely allied bacilli, *B. dys. Shiga*, *B. flexneri*, *B. ambiguus*, *B. alkalescens* and *B. dispar*, are readily differentiated by fermentation reactions. *B. dys. Sonne* appears to be intermediate between *B. dispar* and *B. flexneri*.

3. Subdivision of *B. flexneri* on fermentation reactions is not advisable, but the *flexneri* Z race seems to be characterized by acid production from rhamnose. This character is also strikingly correlated with an inability to attack raffinose when sucrose is fermented.

4. *B. alkalescens* and *B. dispar* form acid from glucose rapidly in a medium containing 1.5% peptone, 0.4% dipotassium phosphate, and 0.2% glucose, then revert rapidly to an alkaline reaction. *B. dys. Shiga*, *B. flexneri* and *B. ambiguus* form acid less rapidly and remain permanently acid or revert slowly.

5. Dyes, such as eosin and methylene-blue, the fuchsin-sulphite indicator, and excess of rosolic acid or china-blue were found to inhibit many strains of dysentery, particularly the *Shiga* type.

6. The following medium is suggested for isolation work:

Distilled water	1,000 c c
Agar	15 gm.
Peptone	10 gm.
Dipotassium phosphate	4 gm.

To each 100 c c of the melted medium add before using:

Lactose, 20% solution.....	5.0 c c
Glucose, 5% solution.....	1.0 c c
Rosolic acid (1.0% in 90% alcohol).....	1.0 c c
China-blue (0.5% in water).....	1.0 c c

The H-ion concentration of this medium, which requires no adjustment of reaction and does not need to be filtered when used on plates, is 7.4 to 7.5.

ON THE PROTECTION AFFORDED TO RED CELLS AGAINST HEMOLYSIS BY EOSIN *

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In 1900 Raab¹ and von Tappeiner² found that if infusoria are placed in a very dilute solution of a fluorescent dye (acridin) and kept in the dark, the organisms are not injured, but on exposing them to diffuse sunlight, death results. The researches of von Tappeiner and Jodlbauer,³ and others have established that the photodynamic effect, associated with fluorescent dyes, is toxic not only for unicellular organisms, but also for enzymes, bacterial toxins, immune bodies, blood cells and even for the higher animals. The effect obtained by placing the photosensitive organisms in the dye and keeping them in the dark for some time is no greater than when the dye is added just before the organisms are exposed to sunlight, and it is immaterial, for the purpose of laking red cells, whether the stain is within the cell or not. Fluorescence is necessary for photobiologic action but the quantitative effect is not proportional to the amount of fluorescence. It is necessary that the fluorescent solution be in intimate contact with the substance on which it is to act; absorption of fluorescent waves alone does not suffice to produce photodynamic effects. The rôle played by oxygen in this phenomenon is still a matter of dispute, but the evidence seems to indicate that its presence is necessary.

Fluorescence is not limited to certain dyestuffs but is a property of many compounds found both in animal and vegetable life and these share with the dyes the common property of sensitizing protoplasm for photodynamic action.

Busck⁴ and later Sellards⁵ found that addition of certain substances, such as blood serum and egg-white, to solutions of photo-

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¹ Ztschr. f. Biol., 1900, 39, p. 524; 1903, 44, p. 16.

² München. med. Wchnschr., 1900, 47, p. 5.

³ Ibid., 1904, 51, p. 737; Deut. Arch. klin. Med., 1904, 80, p. 427; 1905, 85, p. 386; 1906, 86, p. 468 and p. 479; Kudo and Jodlbauer: Biochem. Ztschr., 1908, 13, p. 24; Harzbecker and Jodlbauer: *ibid.*, 12, p. 306; Neuberg: *ibid.*, 13, p. 305; von Tappeiner: *Ergeb. der Physiol.*, 1909, 8, p. 698; Neuberg and Galambos: Biochem. Ztschr., 1914, 61, p. 315.

⁴ Biochem. Ztschr., 1906, 1, p. 425.

⁵ J. Med. Research, 1918, 38, p. 293.